

AMENDMENT

In the Claims:

1. (Currently amended) A method for assaying a sample for an amplification product from a target polynucleotide, comprising:

contacting the sample which is suspected of containing the amplification product with a probe polynucleotide attached to a microsphere that comprises a spectral code comprising a first fluorophore, wherein the first fluorophore is a semiconductor nanocrystal having first fluorescence characteristics;

wherein the amplification product is a polynucleotide and comprises a capture sequence whose complement is not present in the unamplified target polynucleotide at the same position;

wherein the probe polynucleotide comprises a molecular beacon comprising first and second complementary regions and a third region located between the first and second complementary regions;

wherein the molecular beacon can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop;

wherein at least part of the third region is complementary to at least part of the capture sequence in the amplification product, such that the probe polynucleotide can preferentially hybridize to the amplification product and thereby disrupt formation of the stem-loop structure under at least one set of hybridization conditions;

wherein the probe polynucleotide is linked to a first quencher and to a the first fluorophore, wherein the first quencher and first fluorophore are located such that the first quencher can quench a fluorescence emission from the first fluorophore either under a first hybridization state when the probe polynucleotide is not hybridized to the amplification product and the stem-loop structure is formed or under a second hybridization state when the probe polynucleotide is hybridized to the amplification product and the stem-loop structure is not formed, but not under both hybridization states;

wherein the contacting takes place under conditions in which the probe polynucleotide can hybridize to the amplification product, if present;

exciting the first fluorophore by applying a light source to the sample; and
determining the fluorescence emission from the first fluorophore.

2. (Cancelled)

3. (Currently amended) The method of claim 2 1, wherein the semiconductor nanocrystal comprises a core selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaSe, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlSb, AlS, Ge, Si, Pb, PbS, PbSe, an alloy thereof, and a mixture thereof.

4. (Original) The method of claim 3, wherein the core is CdSe.

5. (Cancelled)

6. (Cancelled)

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CDDT. 7. (Original) The method of claim 1, wherein the first quencher quenches the fluorescence emission from the first fluorophore under the first hybridization state.

8. (Original) The method of claim 1, wherein the first quencher quenches the fluorescence emission from the first fluorophore under the second hybridization state.

9. (Original) The method of claim 1, wherein the first quencher is selected from DABCYL, BHQ-1, BHQ-2, BHQ-3, a metal nanoparticle, and a semiconductor nanocrystal.

10-13. (Cancelled)

14. (Original) The method of claim 1, wherein the amplification product is produced at a detectably higher level from a first allele of a locus having at least two alleles.

15. (Original) The method of claim 14, wherein the at least two alleles differ by a single nucleotide.

16. (Original) The method of claim 14, wherein the first allele is an allele associated with an increased risk of a disease or disorder.

17. (Original) The method of claim 14, wherein the first allele comprises an inactivating mutation of a tumor suppressor gene.

18. (Original) The method of claim 14, wherein the first allele comprises an activating mutation of a cellular oncogene.

19-26. (Cancelled)

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27. (Original) The method of claim 1, wherein the sample is simultaneously contacted with a second probe polynucleotide under conditions in which the second probe polynucleotide can hybridize to a second amplification product, if present, from a second target polynucleotide, wherein the second probe polynucleotide comprises a second molecular beacon and a second quencher and a second fluorophore having different fluorescence characteristics than the first fluorophore, wherein a fluorescence emission from the second fluorophore is quenched by the second quencher under only one hybridization state of the second probe polynucleotide with the second target polynucleotide, and wherein a second light source is applied to the sample to excite the second fluorophore, and further determining whether the fluorescence emission from the second fluorophore is quenched or unquenched.

28. (Currently amended) The method of claim 27, wherein the first and second probe polynucleotides are attached to ~~a solid surface~~ the microsphere.

29. (Original) The method of claim 28, wherein a single quencher comprises both the first and second quenchers.

30. (Original) The method of claim 27, wherein the first and second quenchers comprise the same quenching material.

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31. (Original) The method of claim 27, wherein a single light source comprises both the first and second light sources.

32. (Original) The method of claim 27, wherein the first and second amplification products are produced from first and second alleles of a single locus.

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33. (Original) The method of claim 32, wherein the first and second alleles are two alleles of a single nucleotide polymorphism.

34. (Original) The method of claim 32, wherein one of the first and second alleles is an inactivating mutation of a tumor suppressor gene.

35. (Original) The method of claim 32, wherein one of the first and second alleles is an activating mutation of a cellular oncogene.

36. (Original) The method of claim 32, wherein one of the first and second alleles is an allele associated with an increased risk of a disease or disorder.

37-63. (Cancelled)

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